

Assessing Beaked Whale Reproduction and Stress Response Relative to Sonar Activity at the Atlantic Undersea Test and Evaluation Center (AUTEC)

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Award Number: N000141110433
Award Number: N0001412IP20051 / N0001411IP20080
<http://www.bahamaswhales.org>

LONG-TERM GOALS

Atypical mass strandings and behavioral responses of beaked whales have been correlated with exposure to naval sonar (e.g. Simmonds and Lopez-Juraco 1991; Frantzis 1998; Evans and England 2001), highlighting a need to understand the potential physiological impacts to individual whales and if these in turn represent a biologically significant threat to exposed populations. The long-term goal of this study is to assess glucocorticoid levels from blubber biopsies of targeted species, to assess stress levels relative to sonar exposure. Specifically, the project aims to collect biopsy samples at the U.S. Navy's Atlantic Undersea Test and Evaluation Center (AUTEC) in the Andros-AUTEC Operating Area where fleet readiness training involves regular use of mid-frequency active sonars, and compare the levels to those measured in biopsies collected from control populations within the Bahamas region that are less exposed to sonar activity. In parallel, pregnancy states will be ascertained via blubber progesterone levels in both groups of animals to investigate whether there is a relationship between sonar activity, stress measures, and reproductive rates, to assess population-level impacts.

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 2012		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Assessing Beaked Whale Reproduction and Stress Response Relative to Sonar Activity at the Atlantic Undersea Test and Evaluation Center (AUTEC)				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Bahamas Marine Mammal Research Organisation P.O. Box AB-20714 Marsh Harbour Abaco, Bahamas				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES The original document contains color images.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 11	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

OBJECTIVES

The primary objectives of the study are:

- 1) To assess pregnancy rates from progesterone concentrations in blubber biopsies relative to sonar activity, and relate these to stress levels measured from glucocorticoid concentrations in the same tissue for Blainville's beaked whales (*Mesoplodon densirostris*) and sperm whales (*Physeter macrocephalus*).
- 2) To collect photo-identification data to monitor repeated sampling of individuals, construct sighting histories and identify consistent associates as covariates for stress analyses; and to document successful calving events for comparison to hormone-derived pregnancy rates.

APPROACH

The experimental design of this study is based on a population comparison: to compare beaked whale and sperm whale stress levels and pregnancy rates between areas with contrasting sonar activity: at the U.S. Navy's Andros-AUTEC Operating Areas and off the southwest coast of Abaco Island and other regions throughout the Great Bahama Canyon (Figure 1).

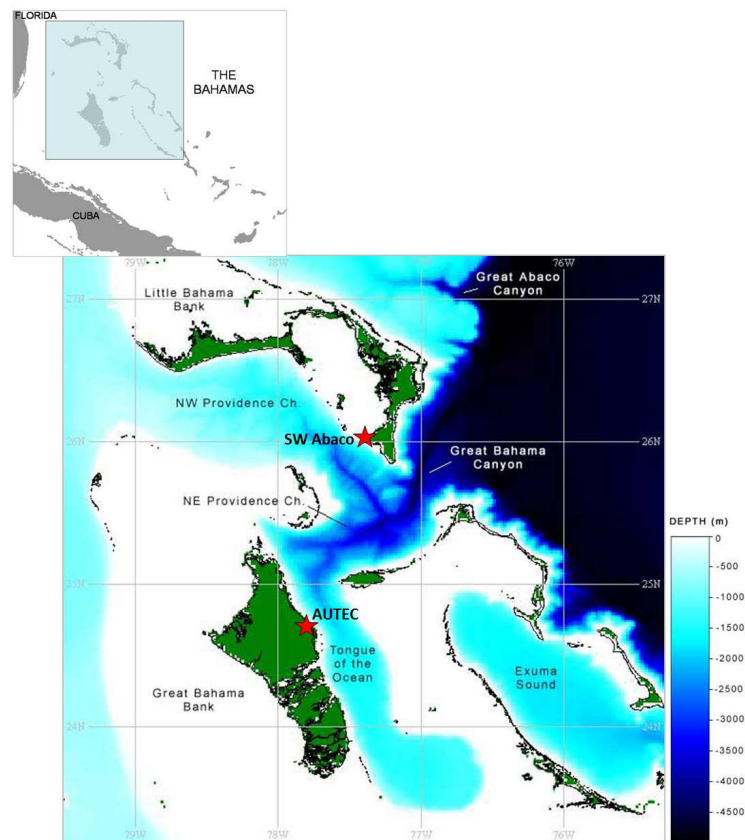


Figure 1. Map of the northern Bahamas showing the location of the two areas where blubber biopsy samples are being collected: AUTEC and SW Abaco. Both lie within the Great Bahama Canyon, a submarine canyon that reaches depths of more than 4000m. Additional sampling is occurring throughout the canyon region.

Stress levels for whales at AUTECH are being evaluated using biopsy samples collected primarily before scheduled Submarine Commanders Courses (SCC) as part of an N45-funded satellite tagging study. Five 15-day field efforts were planned under this study, in Oct/Nov 2011, July 2012 and April/May 2011-13. Reproductive rates will be examined relative to measured stress levels and sonar activity on the range during the preceding months through collaboration with David Moretti at the Naval Undersea Warfare Center. A further 15-day summer sampling effort was conducted at AUTECH in summer 2012 and is planned for summer 2013. In addition, three 15-day field efforts planned in the control area at SW Abaco, spanning May 2011- May 2013, closely matching the timing of the spring SCC at AUTECH. These control samples are being augmented by samples collected during a concurrent annual SERDP-funded sightings-survey around the northern Bahamas, as well as samples collected opportunistically as part of the ONR-funded Bahamas Beaked whale Ecology Study (N000140710120) until that project ended in May 2012. The species targeted are Blainville's beaked whale (*Mesoplodon densirostris*) and sperm whale (*Physeter macrocephalus*).

Using AUTECH's instrumented array of bottom-mounted hydrophones on the Weapons Range, beaked whales and other odontocetes can be monitored and localized in real time by passive acoustic detection of their echolocation clicks (DiMarzio *et al.* 2008). Acoustic technicians from the Naval Undersea Warfare Center relay real-time cetacean localizations using the marine mammal monitoring system at AUTECH and direct observers on a 6.8 m rigid-hulled inflatable (RHIB) to the whales, increasing the opportunities for locating animals and obtaining biopsies. No such array exists off SW Abaco Island; so instead, boat-based surveys are concentrated in areas of known higher density of beaked whales, identified from over a decade of research in the area (Claridge 2006). A hand-held hydrophone is deployed to detect foraging sperm whales. Combined these approaches increase opportunities for finding animals off Abaco Island.

When animals are located, remote biopsy sampling (e.g. Hooker *et al.* 2001) is being used to obtain skin and blubber biopsies. Stress levels are being examined relative to sonar activity by measuring glucocorticoid concentrations in blubber biopsies. Pregnancy state will also be assessed by measuring progesterone concentrations in blubber (Kellar *et al.* 2006; Trego and Kellar 2009), and pregnancy rates will be examined relative to measured stress levels by comparison between samples collected at AUTECH and the control area around Abaco.

Photo-identification data are providing a record of all individuals sighted, and being used to build sighting histories, using new data and the existing BMMRO database. These data will provide information on ranging patterns and demographics that can serve as covariates for analysis of stress patterns. Similarly, photo-identification data will be used to evaluate the stability of individual associations to identify consistent associates that may have similar exposure and stress levels. Longitudinal photo-identification records will also enable documentation of successful calving events, to compare with hormone-derived pregnancy rates.

WORK COMPLETED

During FY12, ONR sponsored two directed field efforts to collect blubber biopsies: 8-22 May at SW Abaco (15 days); and, 1-31 July at AUTECH (31 days, half of which was supported by N45). This effort were augmented by additional time at AUTECH during the N45-supported satellite tagging work from 23 October - 3 November 2011 and from 22 April - 6 May 2012. During the time in the field, vessel surveys were conducted as weather and range access permitted covering over 3,500 km (Figure 2).

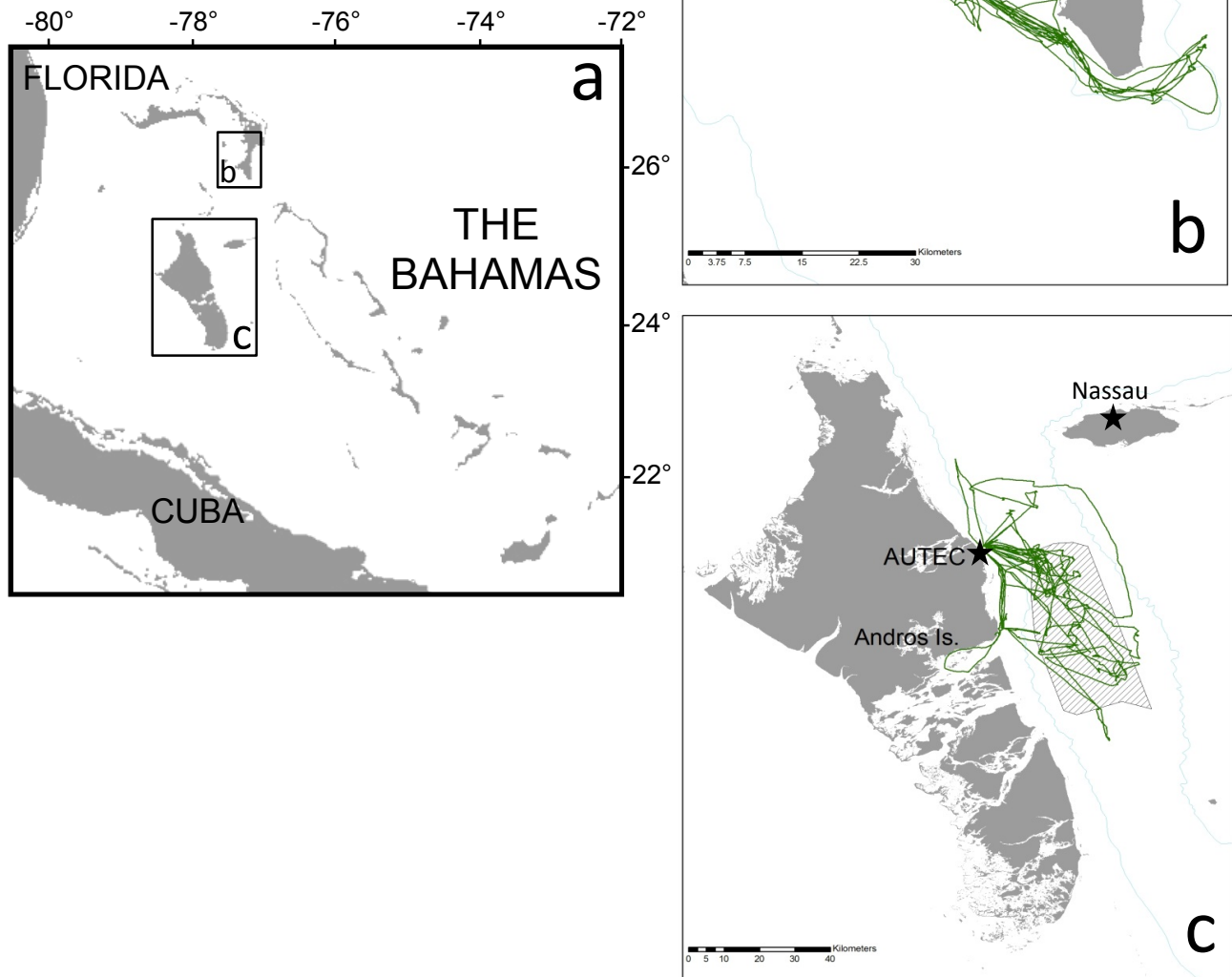


Figure 2. (a) Map of The Bahamas showing the Abaco and Andros study areas. Vessel tracks (in green) show survey efforts supported by ONR off SW Abaco Island (b) and off AUTECH (c) during FY12. The 1000 m isobaths (blue lines) and the outer boundary of the instrumented hydrophone array at AUTECH (hatched area) are also shown.

The field effort at Abaco during May 2012 was extremely productive; weather permitted vessel work during 10 days which covered 886 km of track line, totaling 74.0 hours of search effort. While at AUTECH, despite spending more time in the field including during mid-summer when the sea state was expected to be lower, surveys were conducted less than half of the time the team was on site (22 days of 58 days). However, with the assistance of M3R vectoring the survey team to acoustic detections, when it was calm, efforts were quite productive. At AUTECH the vessel covered a larger area (totaling 2,656 km of track lines), and visual search effort totaled 160 hours. When all efforts were combined, there were 30 Blainville's beaked whale group encounters and 11 sperm whale group encounters (summarized in Table 1).

Table 1. Summary of field efforts when sightings took place of either target species providing opportunity to collect blubber biopsies at both AUTECH and SW Abaco during FY12.

DATE	EFFORT (KM)	EFFORT (HR)	SPECIES	GROUP SIZE	DUR. (MIN)	NO. BIOPSIES
AUTECH – supported by N45						
28-Oct-11	71	4.3	Sperm whale	1	29	1
29-Oct-11	139	9.9	Sperm whale	2	305	0
01-Nov-11	106	9.4	Sperm whale	1	14	0
			Blainville's beaked whale	5	24	0
28-Apr-12	157	7.3	Sperm whale	8	143	1
05-May-12	187	10.8	Sperm whale	1	144	0
06-May-12	171	11.3	Blainville's beaked whale	2	92	0
			Blainville's beaked whale	3	84	0
			Blainville's beaked whale	3	19	0
			Blainville's beaked whale	2	68	0
			Blainville's beaked whale	5	113	0
			Blainville's beaked whale	2	2	0
			Blainville's beaked whale	3	19	0
SW Abaco – supported by ONR						
08-May-12	54	9.4	Sperm whale	15	255	11
11-May-12	123	9.2	Blainville's beaked whale	4	53	3
17-May-12	112	10.9	Blainville's beaked whale	5	124	4
			Blainville's beaked whale	4	12	4
			Blainville's beaked whale	4	19	0
			Blainville's beaked whale	5	98	3
18-May-12	130	10.6	Blainville's beaked whale	5	72	2
			Blainville's beaked whale	5	28	2
19-May-12	150	10.1	Blainville's beaked whale	5	11	1
20-May-12	115	10.2	Blainville's beaked whale	5	84	3
21-May-12	146	10.8	Blainville's beaked whale	6	40	0
			Blainville's beaked whale	5	14	2
			Blainville's beaked whale	4	8	0
			Blainville's beaked whale	5	24	1
			Blainville's beaked whale	5	9	0
			Blainville's beaked whale	3	2	0

Table 1 continued

DATE	EFFORT (KM)	EFFORT (HR)	SPECIES	GROUP SIZE	DUR. (MIN)	NO. BIOPSIES
<i>AUTEC – supported by ONR and N45</i>						
01-Jul-12	78	5.0	Blainville's beaked whale	1	21	1
			Blainville's beaked whale	5	20	1
			Sperm whale	1	16	0
			Sperm whale	1	1	0
07-Jul-12	156	13.3	Sperm whale	2	495	1
08-Jul-12	82	6.1	Blainville's beaked whale	1	24	0
11-Jul-12	71	4.1	Blainville's beaked whale	2	6	1
25-Jul-12	187	11	Blainville's beaked whale	2	62	1
			Blainville's beaked whale	3	15	0
			Blainville's beaked whale	3	63	0
28-Jul-12	157	11.7	Sperm whale	2	17	1
30-Jul-12	119	6.0	Sperm whale	2	135	0

RESULTS

New Data Collected in 2012

During the May 2012 field efforts at SW Abaco we had unprecedented success in obtaining biopsies from both Blainville's beaked whales and sperm whales. During 15 encounters with beaked whales, twenty-four blubber biopsy samples were collected and from a single encounter with 15 sperm whales, eleven samples were taken. [There was an additional sample collected opportunistically at Abaco in November 2011.] In contrast, during the majority of the time at AUTEC, the priority during encounters was to deploy satellite tags instead of biopsy sampling and the smaller collection from this site reflects this. During 16 encounters with Blainville's beaked whales, four blubber biopsy samples were obtained. There were ten sperm whale encounters at AUTEC but group sizes were small (only 1-2 individuals which can make close approaches more difficult) and four biopsies were obtained. The locations where blubber biopsies were collected at both study sites are shown in Figure 3.

Photo-identification analyses are still underway, but here are some preliminary results. Of the 24 blubber biopsies collected from Blainville's beaked whales in SW Abaco, all but one sample were from individuals that have long-term sighting histories from the SW Abaco area. With the exception of young of the year, all age and sex classes, including lactating females, were sampled. Three individuals were sampled twice during the two-week period. Preliminary matching of the sperm whale identification photographs have found three individuals that have been previously sighted in Abaco (Pm003, 043, and 098). Additional information for the biopsy data collected from these whales and the sperm whales are shown in Table 2.

Hormone baselines and QA/QC analysis

In preparation for the tissue processing and hormone measurements of these biopsies, relatively large blubber pieces (8x8cm) from five Cuvier's beaked whales, *Ziphius cavirostris*, and four Hubb's beaked whales, *M. carlhubbsi*, (obtained from fresh strandings and fishery bycatch off California) are being used to optimize extraction and measurement procedures such that they will be more suitable for Blainville's beaked whale tissue. From this analysis, the beaked-whale blubber cortisol and

progesterone values from each species appear to be very similar to one another (Table 3). QA/QC linearity tests were also run with this analysis in efforts to see if each assay if binding to the same antigen in samples and the standards. The parallel segments of the resulting curves matching the true concentrations in the standards versus the serial dilutions of pooled *Mesoplodon carlhubbsi* samples suggest that the assays are binding to the same antigens in either assay. The observed extraction efficiencies are approximately 68% for blubber cortisol and 78% for blubber progesterone, similar to those found for Delphinid blubber. These results, taken together, suggest little alteration will be needed to use our existing procedures on Blainville's beaked whale biopsies. Samples collected during FY11 and FY12 have been imported to the United States and accessioned into the SWFSC tissue archive. The expectation is that once blubber biopsies are obtained from at minimum 30 free ranging individuals with adequate representation of both locations, we will begin laboratory analysis for our two population comparison.

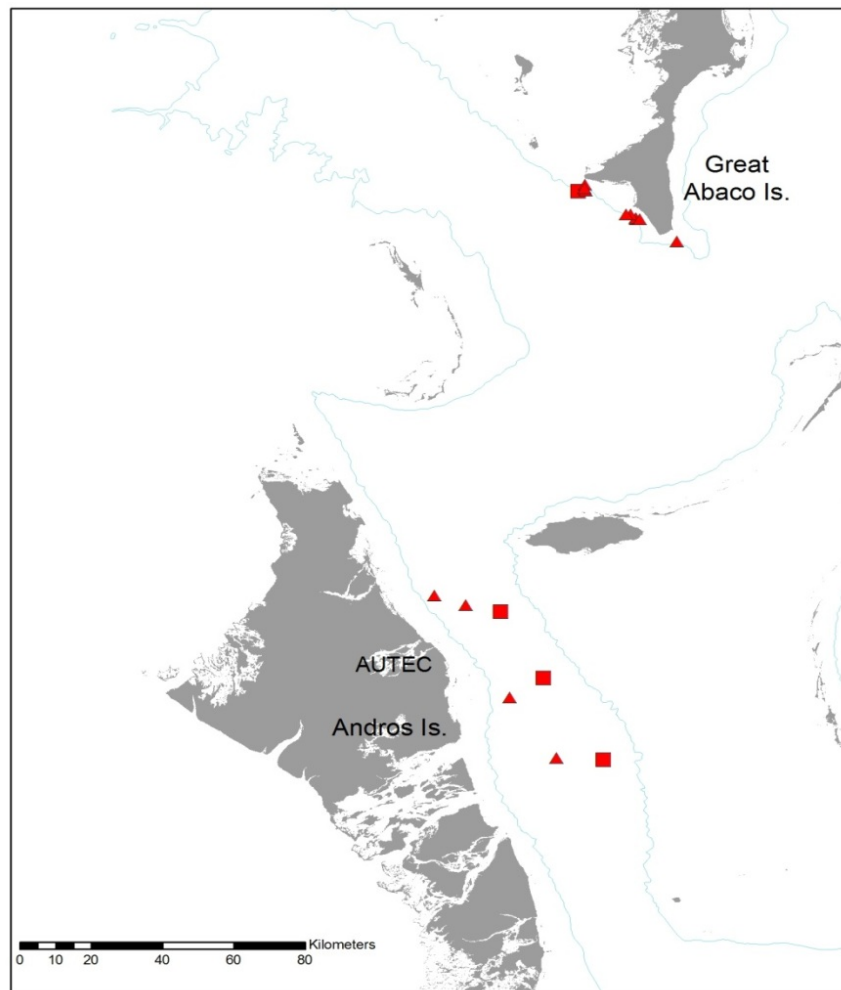


Figure 3. Map of the northern Bahamas showing locations where blubber biopsies were collected from Blainville's beaked whales (red triangles) and sperm whales (red squares) off SW Abaco and AUTECH during FY12. The 1000 m isobaths is shown by the blue lines.

Table 2. Summary of blubber biopsies collected from Blainville's beaked whales and sperm whales at SW Abaco and AUTEK during FY12.

FIELDID	YR	MO	DA	LATD	LATM	LONGD	LONGM	LOCLTY	SEX	AGE CLASS	WHALE ID
Blainville's beaked whale - <i>Mesoplodon densirostris</i>											
120511_Md1b	2012	5	11	25	53.49	77	16.39	SW Abaco	F	AD	Md190
120511_Md3b	2012	5	11	25	53.29	77	16.35	SW Abaco	F	AD	Md107
120517_Md1b	2012	5	17	25	53.59	77	16.85	SW Abaco	F	AD	Md107
120517_Md2b	2012	5	17	25	53.62	77	17.27	SW Abaco	F	AD	Md121
120517_Md3b	2012	5	17	25	53.66	77	17.38	SW Abaco	M	AD	Md242
120517_Md4b	2012	5	17	25	53.49	77	16.83	SW Abaco	M	SA	Md191
120517_Md5b	2012	5	17	25	49.39	77	10.24	SW Abaco	F	AD	Md233
120517_Md6b	2012	5	17	25	49.34	77	10.22	SW Abaco	M	AD	Md069
120517_Md7b	2012	5	17	25	49.33	77	10.23	SW Abaco	F	AD	Md233
120517_Md8b	2012	5	17	25	49.33	77	10.24	SW Abaco	F	AD	Unk.
120517_Md9b	2012	5	17	25	58.28	77	24.27	SW Abaco	F	AD	Md134
120517_Md10b	2012	5	17	25	58.24	77	24.11	SW Abaco	U	JU	Md246
120517_Md11b	2012	5	17	25	58.29	77	23.92	SW Abaco	M	AD	Md211
120518_Md1b	2012	5	18	25	54.22	77	17.23	SW Abaco	F	AD	Md197
120518_Md2b	2012	5	18	25	52.67	77	6.79	SW Abaco	M	AD	Md069
120518_Md3b	2012	5	18	25	54.30	77	17.26	SW Abaco	F	AD	Md135
120518_Md4b	2012	5	18	25	54.21	77	17.18	SW Abaco	M	AD	Md143
120519_Md1b	2012	5	19	25	58.76	77	24.23	SW Abaco	U	JU	Md250
120520_Md1b	2012	5	20	25	53.62	77	16.93	SW Abaco	F	AD	Md091
120520_Md2b	2012	5	20	25	53.41	77	16.82	SW Abaco	F	AD	Md139
120520_Md3b	2012	5	20	25	53.17	77	16.57	SW Abaco	M	AD	Md130
120521_Md1b	2012	5	21	25	53.97	77	17.96	SW Abaco	U	JU	Md245
120521_Md2b	2012	5	21	25	53.93	77	17.95	SW Abaco	U	JU	Md139's calf
120521_Md3b	2012	5	21	25	57.71	77	24.25	SW Abaco	F	AD	Md094
120701_Md1b	2012	7	1	24	50.51	77	42.26	AUTEK	F	AD	Unk.
120701_Md2b	2012	7	1	24	50.51	77	42.26	AUTEK	U	JU	Unk.
120711_Md1b	2012	7	11	24	35.25	77	35.66	AUTEK	F	AD	Unk.
120725_Md1b	2012	7	25	24	25.43	77	27.56	AUTEK	F	AD	Unk.
Sperm whale - <i>Physeter macrocephalus</i>											
111028_Pm1b	2011	10	28	24	43.18	77	29.52	AUTEK	U	SA	Unk.
111115_Pm1b	2011	11	15	26	5.35	77	34.28	SW Abaco	U	AD	Unk.
110428_Pm1b	2011	4	28	24	17.23	77	29.66	AUTEK	U	SA	Unk.
120508_Pm1b	2012	5	8	25	57.91	77	25.78	SW Abaco	F	AD	Pm098
120508_Pm2b	2012	5	8	25	58.55	77	26.08	SW Abaco	F	AD	Pm043
120508_Pm3b	2012	5	8	25	59.60	77	28.06	SW Abaco	F	AD	Pm003
120508_Pm4b	2012	5	8	25	59.94	77	28.39	SW Abaco	F	AD	Unk.
120508_Pm5b	2012	5	8	26	1.54	77	30.44	SW Abaco	U	JU	Unk.
120508_Pm6b	2012	5	8	26	1.52	77	30.47	SW Abaco	U	JU	Unk.
120508_Pm7b	2012	5	8	26	1.63	77	30.89	SW Abaco	F	AD	Unk.

Table 2 continued

FIELDID	YR	MO	DA	LATD	LATM	LONGD	LONGM	LOCLTY	SEX	AGE CLASS	WHALE ID
120508_Pm8b	2012	5	8	26	1.64	77	31.04	SW Abaco	F	AD	Pm043
120508_Pm9b	2012	5	8	26	1.71	77	31.16	SW Abaco	U	JU	Unk.
120508_Pm10b	2012	5	8	26	1.83	77	31.74	SW Abaco	U	SA	Unk.
120508_Pm11b	2012	5	8	26	1.82	77	31.75	SW Abaco	U	SA	Unk.
120707_Pm1b	2012	7	7	24	28.70	77	32.19	AUTEC	U	AD	Unk.
120728_Pm1b	2012	7	28	24	38.08	77	30.67	AUTEC	M	SA	Unk.

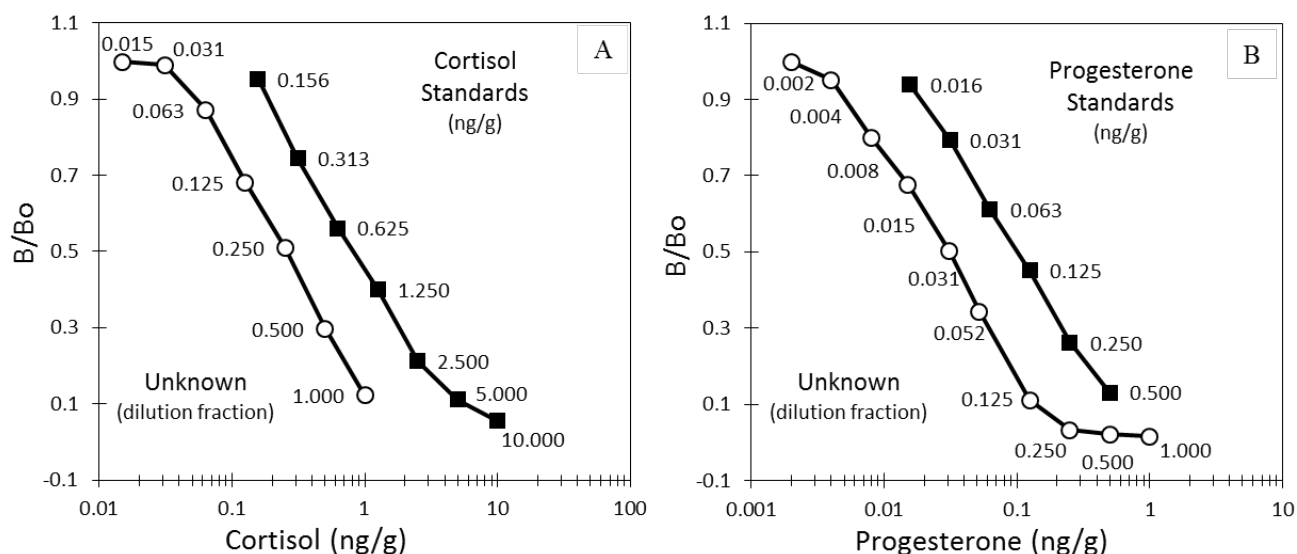


Figure 3. Results from linearity assessment of progesterone and cortisol enzyme immunoassays with *Mesoplodon carlhubbsi* blubber tissue. Serial dilutions of extracts show parallelism with the standards of either assay, an indication that each assay is measuring via the same antigens as standards and therefore suitable for use with the beaked whale blubber tissue.

AUTEC Sperm Whale Hormone Measurements

Blubber cortisol and progesterone have also been measured from the eight sperm whales sampled off AUTEC. No blubber progesterone measurements were consistent with values expected from pregnant cetaceans (i.e., > 40 ng/g), suggesting that none of the sperm whales were pregnant at the time of sampling. In addition, the blubber cortisol levels from these animals were lower than those from the reference beaked whales. Possible explanations for these differences include 1) species/taxa specific differences in baseline blubber cortisol levels (e.g., differences in fatty acid composition in the blubber between sperm whales and beaked whales may result in different cortisol accumulation capacities), 2) sampling process differences (e.g., biopsies float in the water for a short period before they are retrieved potentially disproportionately leaching cortisol), and/or 3) stress-level differences at the time of sampling (e.g., the events resulting in strandings or bycatch may lead to higher circulating cortisol values for a sufficient period such that they are higher in blubber than those in biopsies at the time of sampling). As we continue processing samples from the field we should be able to differentiate which one of these explanations is the primary driver of the observed differences.

Table 3. Summary of blubber steroid measurements from beaked whales and sperm whales, including both reference samples (from bycatch and fresh strandings off California) and unknowns (biopsies from AUTEK).

Species	Type	Location	n	Cortisol (ng/g)	n	Progesterone (ng/g)
				mean ± SE		mean ± SE
Female						
<i>Ziphius cavirostris</i>	Bycatch	California	3	11.29 ± 10.0	3	3.34 ± 1.60
<i>Zc - pregnant</i>	Bycatch	California	1	5.58	1	491.00
<i>Mesoplodon carlhubbsi</i>	Bycatch	California	3	5.20 ± 2.73	3	1.15 ± 0.51
<i>Physeter macrocephalus</i>	Biopsy	AUTEC	6	0.41 ± 0.19	6	0.91 ± 0.35
Male						
<i>Ziphius cavirostris</i>	Bycatch	California	1	1.47		
<i>Mesoplodon carlhubbsi</i>	Bycatch	California	1	1.78		
<i>Physeter macrocephalus</i>	Biopsy	AUTEC	2	0.49 ± 0.21		

IMPACT/APPLICATIONS

Improving our understanding of the population responses of beaked whales relative to sonar usage will aid the US Navy in assessing the potential need for additional mitigation practices for protected marine mammals. In particular, central questions for effective management and potential mitigation are whether sonar use causes detectable physiological stress responses and whether these responses are linked to biologically significant reductions in population health or condition.

This study will build upon ongoing research by Kellar *et al.* assessing stress levels in odontocetes associated with Navy sonar exercises at the Southern California Offshore Range (SCORE). Novel laboratory techniques, recently developed at Southwest Fisheries Science Center (Kellar *et al.* 2006, Kellar *et al.* 2009), are successfully being used to measure steroid hormones in marine mammal blubber indicating that these studies are both realistic and feasible. The combined results of these studies at both AUTEK and SCORE will provide greater power for assessing the extent and magnitude of stress responses in cetaceans exposed to sonar.

RELATED PROJECTS

Monitoring beaked whale movements during the Submarine Commanders Course using satellite telemetry

This project is a collaborative project between the Bahamas Marine Mammal Research Organisation, Southwest Fisheries Science Center and the Naval Undersea Warfare Center (David Moretti). Satellite telemetry is being used to monitor the movements and diving behavior of beaked whales and other odontocete cetacean species on the US Navy's Atlantic Undersea Test and Evaluation Center (AUTEK) range before, during and after sonar exercises in which multiple ships are using their tactical sonars. Field work during this project is providing opportunity to collect biopsy samples and photo-identification data at AUTEK. This project has been supported by the US Department of Defense (OPNAV N45 - Environmental Readiness Division).

Behavioral ecology of deep-diving odontocetes in the Bahamas

This project is examining key aspects of the behavioral ecology of six Department of Defense priority species in The Bahamas. We will integrate data acquired through individual photo-identification, molecular genetics, fatty acid, persistent organic pollutant and stable isotope profiles, satellite telemetry and acoustic recordings to characterize the social structure, residency patterns, reproductive biology, diet, foraging ecology, and population structuring of key cetacean species. Field work during this project is providing opportunity to collect biopsy samples and photo-identification data from throughout the northern Bahamas. The project has been supported by the Strategic Environmental Research and Development Program (US Department of Defense, Department of Energy and the Environmental Protection Agency).

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